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Contribution to the study of the wild House mouse, Genus *Mus* L. (Mammalia, Rodentia, Muridae) in Greece

Study of three populations based on lymphocyte antigen analysis

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Abstract

Typed three Robertsonian wild mice populations from southern Greece, using 16 selected lymphocyte antigens. In all examined mice of the three populations the Thy-1.2 antigen was identified, which is characteristic for the *M. m. domesticus* taxon. On the other hand, the Thy-1.1 allele characteristic for *M. m. musculus* was not found. Moreover, it was indicated that the Ly-1.2 antigen was fixed in these populations while the Ly-1.1 allele was absent. It is already known that although these two alleles cannot be used as markers for the different *Mus* taxa, they can give information about the gene flow between the populations studied.

A polymorphic distribution of the other antigens examined was noted and compared to other European *M. m. domesticus* populations.

It can be concluded from the immunological point of view the studied populations belong to *M. m. domesticus,* and confirms the recent opinions that the Rb populations of *Mus* almost exclusively belong to this taxon.

Introduction

According to many investigators, the entire Greek peninsula is inhabited by two morphologically and biochemically distinct taxa of wild house mice (Bonhomme et al. 1978; Thaler et al. 1981a, b; Bonhomme et al. 1984). One of them belongs to the biochemical group *Mus*-1 and is referred to as *Mus musculus domesticus* Schwarz and Schwarz, 1943 (Darviche and Orsini 1982; Auffray et al. 1990), or as a full species *Mus domesticus* Rutty, 1772. The other taxon belongs to the biochemical group *Mus*-4a, which according to the recent nomenclatorial opinions (Auffray et al. 1990) is *Mus macedonicus* Petrov & Ruzic, 1983.

Many populations of *Mus* in Greece are Robertsonian (Giagia et al. 1987; Tichy and Vucak 1987), i.e., individuals do not have the standard diploid chromosomal number (2n = 40) known for *Mus musculus* but rather karyotypes with 2n = 24, 26, 29, 30 chromosomes. All these variants have occurred by the process of Robertsonian fusion (Robertson and Rees 1916). With respect to the phenomenon of chromosomal repatterning where clearcut instances of speciation processes are very often in progress (Capanna et al. 1977) a clarification of the taxonomic status and inter- and intra-populational relationships of the Rb populations of Greece is needed. Moreover, there is some discussion concerning the presence of Robertsonian populations in subspecies other than *Mus musculus domesticus* (Zima et al. 1990).

Since the classification of different taxa of *Mus* can be undertaken by karyological, genetical and immunological approaches, in this paper we focussed on the immunogenetical approach, based on the study of the major histocompatibility complex (MHC). The MHC is a large chromosome region containing a giant cluster of genes coding for antigens (proteins) which are necessary for functions concerned with the immune response. These

antigens are polymorphic. At least twenty of them are predominantly expressed on lymphocytes and are therefore referred to as Ly antigens (McKenzie and Potter 1979). Kurihara et al. (1985) using monoclonal antibodies for Thy-1, Ly-1, and Ly-2 antigens demonstrated that in eight *Mus musculus* subspecies examined each possessed characteristic phenotype patterns of these antigens. More recently, Figueroa et al. (1986) confirmed the above observations.

Materials and methods

Mice: Wild mice in this survey belong to three different populations. They were trapped at three localities in southern Greece: 1) Kastritsi (Patras) 25 individuals with 2n = 29, 30, 2) Olympia 10 individuals with 2n = 24 and 3) Theba 10 individuals with 2n = 26, 30. Some of the animals were maintained for a certain period in our laboratory and then transported to Germany (Max-Planck-Institut für Biologie, Abteilung Immungenetik, Tübingen). The remainder of animals were transported immediately after trapping.

Antisera and monoclonal antibodies: The monoclonal antibodies used in this survey were kindly provided by Dr. J. Klein, Max-Planck-Institut für Biologie. These antibodies detect 16 lymphocyte antigens. Most of the antigens are expressed on T cells (Ly 1, Thy 1, L_3T_4, $L_{2,3}$) but some are also expressed on B (Ly 1, Ly 5) cells and other cells such as brain cells, epithelial cells and fibroblasts. All monoclonal antibodies used were obtained from ascites fluid produced by inoculation of inbred cell lines into mice. Other antibodies were obtained from supernatants of a cell line grown in culture.

For details of the antisera used in this assay, see Klein (1972), Zaleska-Rutczynska and Klein (1977), Duncan and Klein (1980), and Figueroa et al. (1986).

Complement-dependent cytotoxic assay: For detection of the antigens in this assay single cell suspensions prepared from thymus and lymph nodes were used. Thymus cells were used for the Thy-1.1, Thy-1.2, Ly-1.1, Ly-1.2, Ly-2.1 and Ly-2.2 antigens and the lymph node cells for the detection of Ly-6.2A, Ly-6.2C, Ly-6.2F, Ly-10, Ly-15.2, Ly-18.2, Ly-17.2, Ly-19.2, Ly-28.2. B_2m antigens (Table 1).

Table 1. **Antigens tested and antibodies used to detect them**

Antigen	Antibody	Reactivity of antibody with	Reference
Thy– 1.1	HO.22.1	T cells	Marshak-Rothstein et al. (1970)
Thy– 1.2	5032–1.3	T cells	Mackenzie and Potter (1979)
Ly – 1.1	7.20.6/3	T cells	Hogarth et al. (1980)
Ly – 1.2	C3PO	T cells	Mark et al. (1982)
Ly – 2.1	49–11–1	T cells	Hogarth et al. (1982)
Ly – 2.2	19–178	T cells	Hammerling (unpubl. data)
Ly – 6.2.A	S8.106	T and B cells	Kimura et al. (1984)
Ly – 6.2.C	SKl42.446	T and B cells	Kimura et al. (1984)
Ly – 6.2.F	TUl92.2.10	T and B cells	Klein et al. (unpubl. data)
Ly –10.1	T18/870	T and some B cells	Kimura et al. (1980)
Ly –15.2	8.6.2	T cells	Potter et al. (1981)
Ly –18.2	S8.261	T and B cells	Kimura et al. (1981a)
Ly –17.(20).2	K9–361	T and B cells	Kimura et al. (1981b)
Ly –19.2	K10.6	B cells	Tada et al. (1981)
Ly –28.2	5075–12.1	T and B cells	Hogarth et al. (1984)
B2m	S19/8	T and B cells	Tada et al. (1980)

Two different complement mixtures were necessary: One with the lymph node cells in rabbit normal serum, guinea pig normal serum, and Hank's balanced salt solution (1 : 1 : 3), and the other one with thymus cells in guinea pig normal serum and Hank's balanced salt solution (1 : 4).

To detect individual Ly antigens in wild mice, the lymph node and thymus lymphocytes of these mice were incubated with monoclonal antibodies in the presence of complement and the percentage of killer cells was estimated. The microcytotoxicity test was carried out in Terasaki microplates (IC. A. Greiner and Sohner, Nürtingen, F.R.G.), as previously described (Zaleska-Rutczynska et al. 1983).

The cytotoxicity was evaluated automatically by the propidium idodide method (Bruning et al. 1982).

Results and discussion

As can be seen from Table 2 each population has a different pattern of lymphocyte antigen frequency. For some of these antigens it is characteristic that either they exist in all examined individuals of the three populations (e.g. Thy-1.2) or they are lacking in all of them (Thy-1.1 antigen). The Thy-1.2 lymphocyte antigen is considered as being characteristic for the individuals belonging to the *M. m. domesticus* taxon (Kurihara et al. 1985). Figueroa et al. (1986) confirmed this observations but with one exception. Two of the eight specimens from Costa Rica, expressed the Thy-1.1 antigen although they belong to the *M. m. domesticus* subspecies. The possible explanation for this finding was either that these two animals are progeny of a mouse in which recurrent mutation caused the Thy-1.2, Thy-1.1 conversion [substitution of a single amino acid at position 89 (Williams and Gagnon 1982)] or these two mice may indicate the existence of Thy-1 polymorphism, perhaps present in some populations of *M. m. domesticus*. The presence of the characteristic Thy-1.2 antigen, in all individuals examined here, verifies their systematic position in the *M. m. domesticus* subspecies, a fact that is in agreement with morphological, biochemical and karyological data (Fraguedakis et al. 1986; Chondropoulos et al. 1992; Giagia et al. 1987; Tichy and Vucak 1987). The study of some of the Ly lymphocyte antigens (Table 2) showed that the Ly-1.2 antigen is fixed in all the examined specimens of the

Table 2. **Frequency of Ly antigens in different populations of wild mice**

Population	No. of animals tested	Antigen frequences							
		Thy–1.1	Thy–1.2	Ly–1.1	Ly–1.2	Ly–2.1	Ly–2.2	Ly–6.2A	Ly–6.2C
Kastritsi	25	0	1.0	0	0.96	0.60	0.20	0.36	0.20
Olympia	10	0	1.0	0	1.00	0.60	0.20	0.20	0.10
Theba	10	0	1.0	0	1.00	0.70	0.40	0.40	0
		Ly–6.2F	Ly–15.2	Ly–18.2	Ly–10.1	Ly–19.2	Ly–17.2	Ly–28.2	B_2m
Kastritsi	25	0.20	0.58	0.36	0.20	0.80	0.52	0.40	0.48
Olympia	10	0.66	0.80	1.00	0.40	0.80	0.80	0.60	0
Theba	10	0.70	0	0.70	0.30	0.70	0.70	0.70	0

population from Olympia and Theba and almost all specimens from Kastritsi, while the Ly-1.1 has not been detected in any specimens of these population samples. These results are in agreement with those of Kurichara et al. (1985) and Figueroa et al. (1986) who also mentioned that for different populations of *M. m. domesticus* there is a characteristic mode of fixation of the antigens Ly-1.1, Ly-1.2, i.e., if the individuals of one *M. m. domesticus* population express the Ly-1.1 antigen, the Ly-1.2 is not expressed and vice versa. From these data, we could conclude that although it is not possible to use these antigens as characteristic markers for the taxonomic possition of the specimens, they could be used as markers for determining the migration rates between local populations (Figueroa et al. 1986). The situation with regard to the Ly-2.1 and Ly-2.2 antigens in this study is as follows. The Ly-2.1 allele is very common in the three populations, while Ly-2.2 only appeared in low frequency. This is one more indication of the homogeneity of the Greek populations. Robinson et al. (1984) mentioned that the B_2m^b allele is characteristic for *M. m. musculus,* since it is present in *M. m. musculus* and absent in the *M. m. domesticus* taxon. Figueroa et al. (1986) reported that half of the wild *M. m. domesticus* populations typed by them, reacted with the B_2m-specific antibody. As can be seen from our results, the B_2m^b allele is absent from the two populations and is present in half of the examined specimens of the third one. These results combined with those of Figueroa et

al. (1986) do not confirm the hypothesis that the B_2m^b allele can be used as a marker for the *M. m. domesticus* taxon.

The typing for the remaining Ly antigen revealed a variability in the frequencies among the three populations studied. Although for the majority of the Ly antigens, our results are in agreement with those of Figueroa et al. (1986), some are in contrast. For example, the Ly-28.2 antigen, which the above mentioned authors found to be absent in their collection of wild mice, is present in all our populations. In addition, the Ly-18.2 antigen, which is very rare in the European populations (Figueroa et al. 1986), was found frequently in our populations. The reasons for these fluctuations of Ly antigens among the European *M. m. domesticus* populations is at present unknown and remain to be elucidated. According to the present data we can verify that the three examined populations belong to the same taxon *M. m. domesticus,* a conclusion that is in agreement with correlative morphological data. On the other hand, since the three populations are Rb we agree with the opinion of Zima et al. (1990) that the Rb populations in the *Mus* species are almost exclusively limited to the range of *M. m. domesticus* taxon.

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Zusammenfassung

Beitrag zu Untersuchungen an der wilden Hausmaus, Gattung Mus L. (Mammalia, Rodentia, Muridae) in Griechenland. Untersuchungen an drei Populationen aufgrund von Analysen mit Lymphozyten-Antigenen

In dieser Arbeit werden drei Robertsonsche Wildmaus-Populationen aus Südgriechenland unter Benutzung von 16 ausgewählten Lymphozyten-Antigenen gekennzeichnet. Bei allen untersuchten Mäusen der drei Populationen wurde das Antigen Thy-1,2 identifiziert, welches typisch für das Taxon *M. m. domesticus* ist. Ferner zeigte sich, daß das Ly-1.2-Antigen in diesen Populationen vorhanden war, während das Ly-1.1-Allel fehlte. Wie bereits bekannt, können beide Allele zwar nicht als Marker für die verschiedenen *Mus*-Taxa dienen, sie ermöglichen jedoch Informationen über den Genaustausch zwischen Populationen.

Eine polymorphe Verteilung der anderen untersuchten Antigene wurde angegeben und mit anderen Populationen von europäischen *M. m. domesticus* verglichen.

Aus immunologischer Sicht kann die systematische Stellung der untersuchten Populationen als *M. m. domesticus* bestätigt werden. Das steht in Einklang mit neueren Auffassungen, wonach die Rb-Populationen von *Mus* fast ausschließlich zu diesem Taxon gehören.

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